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Studies of the genetics of populations that were historically endogamous for reasons of geography, religion, or language have contributed substantially to our understanding about hereditary predisposition to cancer. One such population is found in the Netherlands where multiple, prevalent, population-specific founder mutations have been identified . Genetic susceptibility to prostate cancer is being investigated in the Netherlands Cohorts Study (NLCS) on Diet and Cancer, which has identified over 800 cases of prostate cancer among 58,279 male participants. From this study, we have identified 300 cases and 300 controls from among men of comparable age to identify markers near prostate cancer susceptibility genes that are present at higher frequency in the group of men with prostate cancer. We have optimized the methods for analysis of these markers. During the next year, we will apply these methods to analyzing the samples that have been collected.

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INTRODUCTION

This study uses several observations about the genetic basis of prostate cancer to enhance the efficiency of identifying susceptibility genes. 1) Prostate cancer is a multi-step genetic disorder in which some of the observed genetic alterations in prostate cancer cells were acquired through the germline. 2) The chromosomal locations of some of these genes can be identified readily in prostate cancer cells on the basis of their demonstrating loss of heterozygosity. 3) Historically, certain populations have been endogamous causing them to have more genetic homogeneity and to have prevalent founder mutations in some of their disease susceptibility genes. As a result of the population's endogamy, short chromosomal regions have remained identical by descent, leading to recognizable associations of the founder mutations with linked marker alleles (*linkage disequilibrium*). The Dutch represent such a population.

BODY

Task 1. Subject identification. Months 1-12

The project started six months late because of delays in contract negoaitation. Since that time, individuals with prostate cancer have been identified using the Pathology and Cancer Registry database in the Netherlands. The medical histories of each of these subjects have been reviewed, confirming diagnosis of prostate cancer, and noting age and Gleason score at time of diagnosis. Currently, for each subject, tissue blocks are being obtained from non-cancerous tissues (usually lymph nodes) and thick (50 micron) sections are being cut. DNA is being purified from these sections using a protocol optimized in our laboratory and then quantified. To extend the utility of these sections, a technique for whole genome amplification using primer extension preamplification (PEP) was optimized. This technique reproducibly provides approximately 50-fold amplification of the DNA samples. This technique is being applied. Buccal swab samples have been collected from the whole subcohort of the Netherlands Cohort Stduy on Diet and Cancer and DNA has been extracted from these samples. From these samples, 300 will be selected for subsequent analysis. To date, we have collected buccal swabs from 663 male controls from the subcohort of the Netherlands cohort study. We have identified 973 cases of prostate cancer and have collected normal tissue samples for DNA extraction from 101 cases.

Task 2. Development of markers. Months 1-12

A. Markers from regions associated with loss of heterozygosity (LOH) in prostate cancer will be identified and fluorochrome-labeled primers will be synthesized. We have identified microsatellite markers for each of the following chromosomal regions 1q24-q25, 7q31, 8p21-p22, 10q23-q25, 13q14, 16q22, 17p, 17q21-q22, Xq11-q13. Because of uncertainties about relative map positions, we have confined our markers to those which have shown (LOH) in a high proportion of subjects in a single report, to those which show (LOH) in more than one report, or to those whose map positions are known with a high degree of confidence from the GeneMap99 (http://www.ncbi.nlm.nih.gov/GeneMap99) and which are tightly linked to markers that show LOH. In addition, we have added markers for the following chromosomal regions that have shown linkage to prostate cancer susceptibility in families with multiple affected

members, 1q24-25, 1q42-43, and Xq27-28 (Smith, et al., 1996, Cooney, et al., 1996, Gronberg, et al., 1997, Xu, et al., 1998, Berthon, et al., 1998).

B. Standard PCR conditions will be developed for each of these markers. The primer sequences for each of these markers was identified using standard databases (http://www.gdb.org). The predicted sizes of the PCR product alleles were noted and markers yielding products of different predicted sizes were grouped and labeled with one of three different fluorescent dyes (tet, fam, hex). The net effect of this grouping is that multiple markers can either be amplified simultaneous and/or pooled from separate amplifications to minimize the number of electrophoretic runs. Procedures for pooling separate amplification reactions have been optimized.

Different thermostable enzymes were tested for their fidelity for amplifying microsatellites, including AmpliTaq, AmpliTaq Gold, Platinum Taq, Platinum Tsp, and Expand High Fidelity. Among these enzymes, Platinum Tsp (Life Technologies, Gaithersburg, MD) was found to produce the most reliable amplification with the least stutter and the least random addition of an adenine at the 3' end of the PCR product. For each of the markers, different PCR conditions were tested, varying temperature and magnesium chloride concentrations, and the optimum conditions were defined.

KEY RESEARCH ACCOMPLISHMENTS:

Development of DNA databases from cases and cotnrols for genomic analysis.

Development of high-quality, reproducible methods for microsatellite typing

Development of high-quality, reproducible methods for whole genome amplification

REPORTABLE OUTCOMES:

Proposal, "Mentorship Program in Prostate Cancer Genetics" K24 (CA85326-01A1), was funded.

CONCLUSIONS

This works demonstrates the feasibility for high-throughput multiplex microsatellite marker analysis and the feasibility for extending small samples of DNA 50-fold for genetic analysis. It creates the foundations for the analyses that will be performed in the remainder of this study.

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